

**Short Chain Chlorinated Paraffins (SCCP)
Study to Determine Environmental Persistence
Proposed Protocol (April 2005)**

Introduction

The final draft of the updated environmental risk assessment of SCCP (December 2004) included a Marine Risk Assessment (Section 3.3.5) that concluded that SCCP were potentially 'persistent' (P) or 'very persistent' (vP) under the screening criteria defined in the revised Technical Guidance Document. Since the criteria for toxicity (T) and bioaccumulation (B) were also met, it was concluded that a simulation test for biodegradability would be required to determine the half-life for persistence in the environment, and consequently to conclude whether or not the persistency criterion was satisfied.

The updated risk assessment also noted that previous biodegradation studies with SCCP (typically showing significant but low levels of degradation) were conducted at concentrations considerably in excess of the water solubility and that this may have obscured higher degradation rates within the dissolved fraction. Due to the very low water solubility (range from approximately 0.001 to 1 mg/l, depending on chainlength and chlorination level), and the lack of robust, specific analytical methods, the problem of testing at environmentally realistic concentrations, particularly in water below solubility, remains a major difficulty.

The original risk assessment (EC, 2000) estimated that SCCP emissions to the air in the EU represented 0.022% of the total emissions. The updated risk assessment takes into account the cessation of certain uses of SCCP, resulting from marketing and use restrictions, but includes emissions not previously taken into account, in particular the losses over the lifetime and disposal of products containing SCCP. The updated assessment estimates that 5.2 to 6.8% of total EU emissions would be to air. A significant proportion of any emissions to air would be subsequently deposited to surface water and soil, rather than the open sea. Although the updated risk assessment states that the possibility of long-range atmospheric transport of SCCP cannot be excluded, it is clear that the major route to the marine environment is via rivers and estuaries and, therefore, a simulation of degradation in freshwater and/or estuarine systems is considered appropriate. This is considered consistent with the conclusions of the 'STEP' Workshop (Rotterdam, October 2004).

SCCPs have a high affinity for sediment, with a measured organic carbon partition coefficient (K_{oc}) of 199,500. The updated risk assessment estimates regional sediment concentrations to be approximately 7,500 times higher than the corresponding water concentrations (w/w, wet sediment).

Therefore, the proposal is to employ a water-sediment test system (based on OECD Guideline 308). This will allow sufficient quantity of the test substance to be added (to allow degradation to be monitored reliably), the majority of which will partition to the sediment phase, providing a 'reservoir' to maintain a concentration in the water phase at or below the water solubility level.

Test substances

Commercial SCCP consist of a complex mixture of compounds varying in chainlength (C₁₀ to C₁₃) and chlorination level (typically 49 to 70% by weight). In order to monitor mineralisation of the test substance carbon, and due to the difficulties of analysis, ¹⁴C-radiolabelled material will be employed. Since radiolabelled alkane can only be synthesised

at a specified chainlength, it is proposed to test (separately but concurrently) two representative chlorinated alkanes, C10 and C13. This covers both extremes of the commercial product chainlength range. All carbon atoms of each material will be ^{14}C -labelled, in order to detect mineralisation of any part of the molecule.

Because of the small quantities of the radiolabelled alkanes, it will be necessary to dilute each with a quantity (to be defined) of the corresponding unlabelled alkane prior to chlorination. It is proposed to chlorinate each alkane to 65% by weight of chlorine. This is based on 2001-2003 global sales data from a major manufacturer, indicating that 80% of SCCP sold was $\leq 65\%$ chlorinated. Since it is likely that biodegradability will decrease with increasing chlorination level, this is considered to represent a 'reasonable worst case' chlorination value. It should be noted that, due to the small scale of the chlorination process (compared with commercial manufacture), there might be some small deviation from the target value of 65%, but the final level will be determined accurately.

The test concentration selected will depend on the specific activity of the materials, which will depend on the amount of dilution with non-labelled alkanes that proves necessary. Therefore the following protocol refers only to the anticipated test concentration range.

Test system

The test system will comprise a gas flow-through apparatus (as described in Annex 3 of OECD 308) under both aerobic and anaerobic conditions. In summary, this consists of replicate sediment-water systems (water:sediment ratio of 3:1 to 4:1) through which is passed a flow of air (for aerobic systems) or nitrogen (for anaerobic systems). The gas flow will flush volatile breakdown products into downstream traps, for intermittent determination of evolved radiolabelled CO_2 /methane and other volatile transformation products, with analysis of the sediment and water at intervals to determine a mass balance. The test vessels will be incubated at $20 \pm 2^\circ\text{C}$, in the dark. The testing of fully anaerobic conditions is subject to agreement that degradation under these conditions is considered relevant to the EU PBT criteria [see Note 1].

Sediments

Two sediments (freshwater and/or estuarine) will be tested for both the aerobic and anaerobic tests, collected (following the principles described in OECD 308) from sites with no known history of contamination with SCCP and distant from other significant sources of chemical contamination. The characteristics of the sediments, in terms of organic matter content and ambient salinity require agreement; if both were to be estuarine, it is proposed that one would be from the upper estuary (predominantly low salinity) and one from the lower estuary (predominantly high salinity). As described in OECD 308, the two sediments should differ in organic carbon (OC) content by at least 2%; it is proposed that OC contents of 2-5% and 5-10% are employed. If possible, the anaerobic sediments will be sampled from the same general locations as the aerobic sediments. For the anaerobic study, the sampling and holding of the sediments will be designed to maintain anaerobic conditions throughout. Overlying water for the test will be collected at the same time from the same locations. The sediments will be characterised for particle size and organic carbon content and the waters for pH, hardness, alkalinity, conductivity and salinity, as appropriate.

Sediment spiking

Contrary to the preference given in OECD 308, it is not considered feasible to add SCCP directly to the water phase of the test system. Due to its low water solubility, it would not be

possible to add sufficient material (to allow analytical detection) in this way without exceeding the water solubility by more than an order of magnitude. Therefore, it is proposed that the SCCP is spiked into the sediment phase. A solution of the substance in a volatile organic solvent (eg acetone) will be used to treat a proportion (ca 5-10% by weight) of the sediment that has been dried, and the acetone allowed to evaporate prior, to mixing with the remainder of the sediment. This method of spiking will not be consistent with the one to two week acclimation phase (under test conditions but without test substance) recommended in OECD 308. However, the stabilisation of pH, oxygen concentration, redox potential etc will be monitored during the first two weeks on control vessels treated in the same manner. The distribution of the substance between the sediment and water phases will be determined by radiochemical analysis at time zero.

Experimental design

A single concentration of each test substance will be tested. This would be selected based on the specific activity of the radiolabel, to ensure any that significant degradation could be detected reliably. If possible, this would be in the range of approximately 1 to 10 mg/kg dry weight, which would be environmentally realistic based on the predictions of the risk assessment.

Sufficient replicates of each treatment will be tested to allow the sampling schedule described below. The nominal duration of the study will be 14 weeks (98 days) but, dependent on the results, the experimental design would allow for a longer duration with additional sampling points.

Sampling

Quantities of trapped radiolabelled CO₂/methane (and other organic volatile degradation products) will be determined at regular intervals (7 occasions, approximately every 2 weeks) and the traps on the vessels renewed. Duplicate vessels will also be sacrificed on Day 0 and at least four further occasions for determination of the concentrations of radioactivity in the sediment and water (and for mass balance determinations). The timing of these sacrificial analyses will depend on the rate of evolution of ¹⁴CO₂/methane that is observed.

Mineralisation to CO₂/methane will be the principal measure of biodegradation. However, on certain occasions when vessels are sacrificed for mass balance determination, samples or extracts of water and sediment may be retained for determination of the parent compound and potential metabolites, as required by the sponsor; however, no specific analytical techniques can be defined at the present time. Although recently-developed methods (using gas chromatography-high resolution mass spectrometry (GC-HRMS) with electron capture negative ionisation (ECNI)) have improved the analysis of SCCP, there is no data on the utility of such methods for detecting or identifying metabolites of SCCP biodegradation.

Treatment of results

The recovery of radioactivity as evolved CO₂/methane, and the mass balance on sacrificed vessels, will be reported as percentages of the applied radioactivity. The half-life and its confidence limits will be calculated using regression analysis or other techniques, as appropriate to the data obtained, and as described in OECD 308.

Notes

(1) The aerobic system described in OECD 308 is designed to provide aerobic overlying water and surface sediment layer, the subsurface sediment being undisturbed and allowed to become anaerobic. A possible

alternative to the use of a fully anaerobic treatment would be to remix these aerobic and anaerobic zones at regular intervals (eg weekly), to allow cycling of aerobic and anaerobic conditions and better exchange of test substance/degradates between the sediment layers. This may be important if dehalogenation (which might be expected to occur under anaerobic conditions) was necessary before aerobic degradation of the paraffin could occur, and would better simulate the real environment in which sediment is cyclically buried and re-suspended.